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STUDIES ON PATIENTS CONVALESCENT FROM FRACTURE

III. The Urinary Excretion of Calcium and Phosphorus[1]

JOHN EAGER HOWARD, M.D., WILLIAM PARSON, M.D., AND ROY S. BIGHAM, JR., M.D.

With the technical assistance of Harry Eisenberg and Kay Eisenberg Stein

From the Department of Medicine of the Johns Hopkins University and the Johns Hopkins Hospital

INTRODUCTION

In the course of convalescence from skeletal injuries, urinary lithiasis is a frequent complication (1). At a recent symposium on kidney stones held at the Newton D. Baker General Hospital, Major J. J. Joelson (2) reported that over a 7-month period 31 cases of nephrolithiasis had been observed in recumbent patients at the Crile General Hospital, an incidence of 2% of all orthopedic admissions and 7% of the cases of fractured femur. Major Frank C. Hamm (3) reported 62 instances of nephrolithiasis that he and Major Leader had seen in consultation among orthopedic cases at Fletcher General Hospital and Wakeman General Hospital.

Roentgenographic studies of limbs encased in plaster or in splints for long periods of immobilization usually disclose marked rarefaction of the bones. If the loss of lime salts entailed in such rarefaction should be removed from the body largely by way of the urine, the added increments of calcium and phosphorus might prove to be factors of importance in the incidence of stone formation. With this in mind, the movement of calcium and phosphorus, with especial attention to the amounts and concentrations of these elements in the urine, was studied in a group of 17 patients at bed rest with complete leg and partial body cast.

Thirteen patients had suffered femoral or tibial fractures. Four patients were studied before and after femoral osteotomy for slipped epiphyses. All of the patients of this group were males; their ages

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Johns Hopkins University.

ranged from 14 to 64 years. All were in a good state of nutrition and manifested no abnormalities other than those associated with the skeletal injury for which they were hospitalized. All observations were made on the Metabolism Ward of the Johns Hopkins Hospital. Orthopedic management was carried out by the orthopedic staff under the direction of Dr. George E. Bennett.

METHODS

Diets were prepared in the Metabolism Ward diet kitchen under the direction of Miss Virginia Reidt. Urine was collected directly into bottles containing hydrochloric acid and toluol. Analyses were made on collections covering 3-day periods. Once or twice a week blood was drawn from brachial veins with minimal stasis in the fasting state, and the following determinations were made: hemoglobin (Sahli-Helige), hematocrit (4), serum protein[2], serum chloride (8), serum CO_2 (9, 10), serum calcium (11), serum inorganic phosphorus (12), serum phosphatase activity (13, 14). Urinary calcium was determined from aliquots covering periods of 1 to 5 days (15). Urinary phosphorus determinations were made according to Fiske and Subbarrow (12) on period aliquots. The dietary content of food was calculated from Sherman (16) with some check analyses in our own laboratory. The figures used for milk, however, were determined by our own analyses.

RESULTS

A steady rise in the urinary content of calcium was observed in patients who suffered fractures. Maximal urinary excretion of calcium was reached approximately one month after injury, and this peak excretion was maintained for an indefinite period thereafter, usually falling at the time the orthopedists allowed the patient to begin mobilization, but in some instances beginning to fall at 60 or 70 days, prior to mobilization. When the urinary output of calcium is charted (Chart I), the curve is seen to have a steady rise and then a sort of plateau is reached, much like the stadium incrementi and stadium

[2] At first during this study serum protein determinations were made-refractometrically (5); soon we changed to a falling drop method (6); and later the determinations were made by a MacroKjeldahl method (7).

fastigium of the temperature chart of patients suffering from typhoid fever. Although the quantity of calcium excreted by way of the urine varied from patient to patient in both the early period after fracture and during the fastigium phase, in every instance the *pattern* of rise to maximum excretion and subsequent maintenance of this level was similar.

The calcium content of the urine in patients after femoral osteotomy followed a similar pattern (Chart II), although maximal excretion of calcium was reached much sooner and, hence, the stadium incrementi was much shorter.

Previous reports from this clinic (17, 18) have dealt with the metabolism of nitrogen after fracture and osteotomy, confirming the earlier

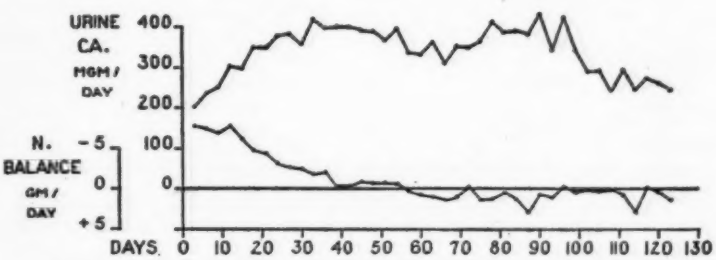

CHART I. URINE CALCIUM VS. NITROGEN BALANCE AFTER FRACTURE

observations of Cuthbertson (19). In vigorous healthy patients who have suffered fracture, there occurs a rapid breakdown of protein, manifested by the urinary excretion of large quantities of nitrogen. This process of increased protein breakdown reaches a maximum at about 5 to 7 days after injury and gradually subsides in from 25 to 40 days. The relationship of the process resulting in increased excretion of calcium in the urine to the protein catabolism reaction is of interest. Maximal excretion of calcium was reached in every instance at a time when the protein catabolism reaction had waned; i.e., to the point where nitrogen equilibrium was approximated with diets containing both calories and protein in amounts usually needed for nitrogen balance at ordinary bed rest without injury (18). Chart I depicts the average excretion of calcium in the urine of the 13 patients with fracture and the average nitrogen balance curve of these same patients.

A reciprocal relationship between the peak intensity of urinary excretion of calcium and the protein catabolism reaction is further suggested by similar observations on the patients who were subjected to osteotomy. In this group the period of increased protein breakdown and the intensity of the reaction were shorter (17). Maximum urinary calcium excretion was reached sooner than in the patients with fracture (Chart II). Here again subsidence of the protein catabolism reaction in each instance coincided with the point of maximum diuresis of calcium, i.e., the beginning of the fastigium phase.

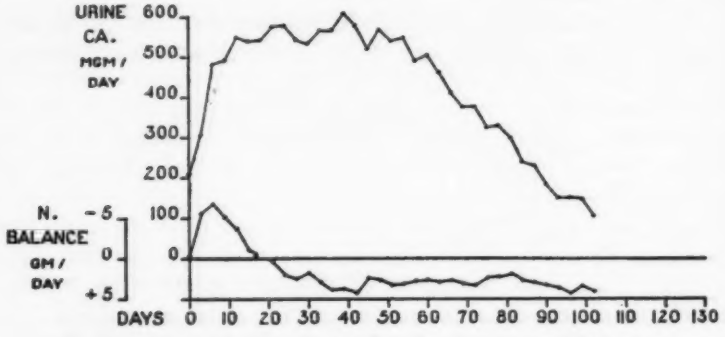

CHART II. URINE CALCIUM VS. NITROGEN BALANCE AFTER OSTEOTOMY

The type of injury suffered, i.e., whether the fracture was simple or compound, did not appear to be a determining factor in the quantity of calcium excreted in the urine during the fastigium phase. The average content of calcium in the urine of patients with simple fracture in the fastigium phase was 513 mgm. per day; for the patients with compound fracture 503 mgm. per day.

The average age of this group of patients was 33.7 years. Although the lowest value of maximal urinary excretion of calcium occurred in the oldest individual, the next to the highest excretion occurred in a patient only 7 years younger. As seen in Table I there is no regular relationship between the amount of calcium excreted in the urine and the age of the patients.

In those instances where exact height and weight of the patients were known, the urinary calcium excretion per 1.73 square meters of

TABLE I

Type of Fracture, Age and Maximum Urinary Calcium Excretion in 17 Cases

PATIENT	TYPE OF FRACTURE	NO. OF BONES INVOLVED	AGE	MAXIMUM URINARY Ca
				mgm./day
Be.	Simple	Single	15	670
Je.	Simple	Single	14	635
Wh. 1	Simple	Single	16	525
Wh. 2	Simple	Single	16	630
Ha.	Simple	Single	50	520
Ne.	Simple	Single	57	475
Bu.	Simple	Single	31	385
Ba.	Simple	Single	54	640
Ra.	Simple	Multiple	28	325
Wo.	Simple	Multiple	24	445
Ma.	Simple	Multiple	27	400
Average.....			31.6	513.6
Wa.	Compound	Single	24	485
Jo.	Compound	Single	32	620
Br.	Compound	Single	39	605
Fr.	Compound	Multiple	26	510
Do.	Compound	Multiple	61	295
Ty.	Compound	Multiple	41	505
Average.....			37.2	503.3

TABLE II

Maximum Urinary Calcium Related to Surface Area of Patient

PATIENT	SURFACE AREA	MAXIMUM URINARY CALCIUM	MAXIMUM URINE CALCIUM/1.73 SQ. M.
	sq. m.	*mgm./day*	*mgm./day*
Wh.	1.48	630	735
Je.	1.69	635	650
Be.	1.72	670	675
Fr.	1.76	510	500
Ha.	1.85	520	485
Jo.	1.95	620	550
Wo.	2.02	445	380
Ne.	2.04	475	400
Ra.	2.08	325	270
Wa.	2.09	485	400

surface area was determined, and no correlation could be seen between the size of the individual and the magnitude of calcium diuresis during the fastigium phase. (Table II)

Since individuals doing heavy muscular work are known to have greater bone structure than those who take little exercise, the cases in this study were classified (Table III) into two groups; 9 having done

TABLE III

Maximum Urinary Calcium Relative to Previous Activity of Patient

PATIENT	ACTIVITY PRIOR TO INJURY	MAXIMUM URINARY CALCIUM
		mgm./day
Be.	Restricted	670
Je.	Restricted	635
Wh. 1	Restricted	525
Wh. 2	Restricted	630
Ne.	Light work	475
Ra.	Light work	325
Wo.	Light work	445
Fr.	Unemployed	510
Average		527
Ha.	Laborer	520
Bu.	Laborer	385
Ba.	Laborer	640
Ma.	Laborer	400
Wa.	Laborer	485
Jo.	Laborer	620
Br.	Laborer	605
Du.	Laborer	295
Ty.	Laborer	505
Average		495

manual labor, and 7 light work or having been relatively inactive (the patients who underwent osteotomy had been on markedly restricted activity because of their painful hips). The average maximum excretion of calcium in the urine of the laborers was 495 mgm. per day, for the inactive group 527 mgm. per day. There is thus no support in these data for the suggestion that patients with dense bones will lose more calcium in the urine when immobilized than will those patients who have been relatively less active.

During the prolonged fastigium phase, when the urinary output of calcium remained relatively constant, various changes in the regimens were carried out to determine their possible influence on the excretion of calcium in the urine. For the most part water was allowed the patients ad libitum, and their intake was usually 2500 to 3500 cc. total fluids per day. When liquids were abruptly curtailed in patient Wh. to 750 cc. per day for 3 days, the total daily excretion of calcium in the

TABLE IV

Urine Calcium Related to Administration of Alkalinising Agents

PATIENT	DAYS AFTER INJURY	CALORIES	DIET N	DIET Ca	DIET P	ALKALI DAILY	URINARY CALCIUM
			gm./day	*mgm./day*	*mgm./day*		
Wh.	47–49	2600	18	2000	2300	0	565
	50–52	2600	18	2000	2300	0	600
	53–55	2600	18	2000	2300	$NaHCO_3$ 6 gm.	585
	56–58	2700	17	2000	2300	6 gm.	530
	59–61	2700	17	2000	2300	6 gm.	485
	62–64	2600	18	2000	2300	6 gm.	570
	65–67	2600	17	2000	2300	6 gm.	510
	68–70	2500	17	2000	2300	0	515
	71–73	2500	17	2000	2300	0	495
Do.	73–79	2700	17	2000	2100	0	235
	80–86	2700	17	2000	2100	NaCitrate 6 gm.	245
	87–107	2700	17	2000	2100	NaCitrate 12 gm.	240
	108–129	2700	17	2000	2100	$NaHCO_3$ 9 gm.	220

urine remained unchanged though the urine volume was reduced from 1500 cc. to 500–600 cc. per day. The urine contained more than 550 mgm. calcium per day at this time and neither calcium casts nor RBC were noted in the urine, which was acid throughout.

Alkalis were administered to two patients during periods of maximum urinary excretion of calcium (Table IV). Diets were kept constant in each instance throughout the period of study. To Wh., on a diet of 2300 calories, 2300 mgm. phosphorus and 2000 mgm. calcium, 6 gms. sodium bicarbonate were given daily in three divided doses

after meals for five periods of three days each. No effect on the output of calcium via the urine was observed.

To Do.—diet 2700 calories, 2100 mgm. phosphorus and 2000 mgm. calcium—was given 12 gms. sodium citrate for 20 days and 9 gm. sodium bicarbonate for 21 days without detectable effect on the urinary content of calcium. In both these patients, the urine became alkaline under the therapy; but no albumen or abnormal elements in the urinary sediment appeared.

Sulfadiazine and sulfathiazole were administered p.o. to two patients in doses of 4 gms. for the first dose and thereafter 1 gm. every 6 hours for periods of one week. These observations were made during the fastigium phase of urinary excretion of calcium when the patients were afebrile and had been on constant full diets for at least two weeks. Alkalis were not administered with the sulfonamides. No change in the amount of calcium excreted in the urine was observed from the administration of these drugs, nor were abnormal elements noted in the urinary sediment.

Dietary Factors

In order to evaluate the relationship between the amount of ingested calcium and the calcium content of the urine, abrupt changes in the calcium of the diet from high to low or in reverse order were made in several instances (Table V). The caloric and nitrogen ratios of the diets were always approximately 1000 calories per 6.25 gms. so that the caloric content may be readily ascertained from the amount of ingested nitrogen.

In case Be., at the bottom of Table V, the dietary shifts were made both before and after the period of nitrogen wastage. From the fifth to the eighth day after his manipulation for slipped epiphysis, the patient's diet contained 11 gms. nitrogen per day (approximately 1760 calories) and 1400 mgm. calcium. There was a negative nitrogen balance averaging 6.5 gms. per day and the average daily excretion of calcium in the urine, which would not have been expected to have reached its peak, was 512 mgm. In the next six days the daily dietary calcium was sharply reduced to 325 mgm. with little change in dietary nitrogen. The patient's urinary excretion of calcium *rose* slightly; the nitrogen balance was still negative. During the succeeding six

TABLE V

Effect of Change in Dietary Calcium Intake on Urinary Calcium after Trauma

PATIENT	DAYS AFTER INJURY	CALORIES	DIET N	DIET Ca	URINE Ca	N BAL.	CHANGE IN N INTAKE	CHANGE IN DIET Ca	CHANGE IN URINE Ca
			gm./day	mgm./day	mgm./day	gm./day	gm./day	mgm./day	mgm./day
Wh.	15–17	1800	12	300	563	−1			
	18–20	1850	13	300	576	+1	+1	0	+13
	21–23	2500	17	2000	541	+3	+4	+1700	−35
	24–26	2600	18	2000	628	+2	+1	0	+87
Wh. 2nd oper.	9–11	1800	11	300	450	−3			
	12–14	1900	12	300	525	−1	+1	0	+75
	15–17	1800	12	300	565	−1	0	0	+40
	18–20	1800	13	300	575	+1	+1	0	+10
	21–23	2500	17	2000	540	+3	+4	+1700	−35
	24–26	2500	18	2000	630	+1	+1	0	+90
	27–29	2500	18	2000	545	+1	0	0	−85
	30–32	2500	19	2000	565	+3	+1	0	+20
Ha.	34–36	1600	12	300	425	−5			
	37–39	1750	12	300	405	−4	0	0	−20
	40–42	2750	17	2000	520	−2	+5	+1700	+115
	43–45	2700	16	2000	465	−1	−1	0	−55
Jo.	31–33	1500	8	200	495	−4			
	34–36	1600	7	200	485	−4	−1	0	−10
	37–39	2450	16	2000	620	+1	+9	+1800	+135
	40–42	2600	17	2000	510	+1	+1	0	−110
	43–45	2400	16	2000	510	0	−1	0	0
	46–48	2600	16	2000	490	+2	0	0	−20
	49–51	1700	12	250	440	−1	−4	−1750	−50
	52–54	1700	11	250	460	−2	−1	0	+20
Bu.	77–79	2700	17	1950	280	0			
	84–86	2700	16	1950	230	+3	−1	0	−50
	91–93	1750	12	550	235	+1	−4	−1400	+5
	98–100	1500	12	550	230	0	0	0	−5
Ne.	39–41	1800	12	300	345	−2			
	42–44	1800	12	300	340	−1	0	0	−5
	45–47	1700	11	2000	475	−4	−1	+1700	+135
	48–50	1800	11	2000	415	−3	0	0	−60
	51–52	1800	11	2000	455	−1	0	0	+40
	53–55	1700	11	2000	435	−1	0	0	−20
	56–58	1800	12	500	355	0	+1	−1500	−80

TABLE V—*Continued*

PATIENT	DAYS AFTER INJURY	CALORIES	DIET N	DIET Ca	URINE Ca	N BAL.	CHANGE IN N INTAKE	CHANGE IN DIET Ca	CHANGE IN URINE Ca
			gm./day	*mgm./day*	*mgm./day*	*gm./day*	*gm./day*	*mgm./day*	*mgm./day*
Ne.—*Cont.*	59–61	1600	11	500	255	−2	−1	0	−100
	62–64	2000	12	300	260	−2	+1	−200	+5
	65–67	1900	12	300	305	−3	0	0	+45
Be.	5–8	2000	11	1400	510	−7			
	8–14	2200	13	325	580	−5	+2	−1075	+70
	14–20	2400	13	2000	575	−1	0	+1675	−5
	20–26	3300	20	2000	570	+5	0	0	−5
	26–32	2200	11	325	535	0	−9	−1675	−35

days (14–20 after injury), with the same nitrogen intake as before, nitrogen equilibrium was established. During this period the bulk of the dietary nitrogen was given as milk, thus providing 1675 mgm. more calcium per day than in the preceding period. The change in the output of urinary calcium was negligible. In the period covering days 20–26 after injury, he was still fed 2000 mgm. calcium as milk and, in addition, the caloric and nitrogen content of the diet were sharply raised. Though strongly positive nitrogen balance resulted (partly, too, because the protein catabolism reaction was over), there was no change in the calcium output via the urine. In the last metabolic period on this patient represented in the table (days 26–32 after injury), the dietary provision of nitrogen, calories and calcium were all reduced together, the calcium intake having been only 325 mgm. per day. Urinary excretion of calcium was affected but little, certainly no more than was seen repeatedly from week to week in many other patients who were on constant diets.

Dietary shifts in calcium intake of a similar nature made on other patients are also represented on Table V. These latter observations were all made *after* the period of nitrogen wastage and thus during the fastigium period of urinary excretion of calcium. In most instances, relatively inconsequential changes in the content of calcium in the urine followed the large shifts in dietary calcium. It is perhaps of significance that in all the instances so far cited the largest moiety of

the dietary calcium was provided as milk; hence, provision of larger or smaller quantities of phosphorus were made coincidently.

In one patient, Ne., a large increase in dietary calcium was provided as calcium lactate[3], given in water after meals (Table VI). For 11 days, from the 44th through the 55th days after his fracture was sustained, the patient received 1560 mgm. calcium per day. The average daily excretion of calcium rose from 345 to 450 mgm. per day during

TABLE VI

Effect of Calcium Lactate on Urinary Calcium Excretion

Patient Ne.

DAYS AFTER INJURY	CALORIES	DIET N	Ca INTAKE	URINE Ca	CHANGE IN Ca INTAKE	CHANGE IN URINE Ca
		gm./day	*mgm./day*	*mgm./day*	*mgm./day*	*mgm./day*
39–41	1800	12	300	345	—	—
42–44	1800	12	300	340	0	−5
45–47	1800	11	2000*	475	+1700	+135
48–50	1800	11	2000*	420	0	−55
51–52	1800	11	2000*	455	0	+35
53–55	1800	11	2000*	435	0	−20
56–58	1800	12	500	355	−1500	−80
59–61	1800	11	500	255	0	−100
62–64	1800	12	300	260	−200	+5
65–67	1800	12	300	305	0	+45

*Calcium increased as calcium lactate 12 gm. daily.

these periods, and fell back to 260 mgm. per day in the two periods after withdrawal of calcium lactate. Addition and subtraction of dietary calcium in the form of lactate is thus reflected by somewhat greater

[3] Calcium lactate U.S.P. XII ($Ca(C_3H_5O_3)_25H_2O$) = 13% calcium)

To another patient, not included in the table, calcium lactate 4 gms. t.i.d. was given for one week. The total ingested calcium was 2000 mgm. daily and the phosphorus 2300 mg. At the end of the week numerous RBC and calcium casts appeared in the urine and the administration of lactate was stopped, with prompt disappearance of the urinary abnormalities. During the period of lactate administration the urine pH rose from 6.0 to 7.0 and the serum CO_2 from 25 to 30 MEQ. per liter and the chloride fell from 106 to 100 per liter. There was no change observed in the amount of calcium excreted in the urine during this period, the urinary content of calcium being approximately 575 mgm. per day before, during and after the period of calcium lactate administration.

changes in the urinary excretion than when the dietary increments were in the form of milk.

The effect of varied doses of vitamin D_2 (calciferol) on the urinary excretion of calcium is represented in Table VII. Three patients with fractures were studied, all after the period of nitrogen wastage was past. It may be observed that 5,000 units of calciferol did not affect the urinary excretion of calcium, either during a period of high calcium administration (days 75 to 88) or during a period of lower calcium

TABLE VII

Effect of Calciferol on Urinary Calcium after Trauma

PATIENT	DAYS AFTER INJURY	CALCIFEROL UNITS—DAILY	CALORIES	DIET Ca	URINE Ca
				mgm./day	*mgm./day*
Bu.	61–74	0	2700	1950	230
	75–88	5000	2700	1950	255
	89–109	5000	1800	550	235
	110–123	0	2500	2000	245
	124–151	50,000	2500	2000	280
	152–172	0	2500	2000	285
Br.	44–64	170	2700	1950	510
	65–85	5000	2700	1950	575
	86–99	15,000	1800	500	565
Do.	30–64	0	2700	2000	250
	65–99	5000	2700	2000	255
	100–127	15,000	2700	2000	220

administration (days 89 to 109). 50,000 units daily of calciferol associated with a high calcium administration likewise reflected a negligible increase in urinary calcium (days 124 to 151). Similar insignificant changes in urinary excretion of calcium occurred during administration of 5,000 and 15,000 units of calciferol in the other two patients shown in Table VII. In these studies on calciferol, the shifts in calcium administration were all in the form of milk, and during the periods of lower calcium administration the nitrogen and caloric contents of the diets were also appreciably lower.

Urinary Phosphorus

The excretion of inorganic phosphorus in the urine has been studied in the same group of patients. Variations in the urinary content of inorganic phosphorus were in general much wider than in the case of calcium and were found to be more susceptible to change as the result of dietary shifts. No effect on the output of phosphorus in the urine was detectable from administration of sulfathiazole or sulfadiazine, from the administration of sodium citrate or sodium bicarbonate, from restriction of water or administration of calciferol in doses of 5,000, 15,000 or 50,000 units per day. At the time these observations were made on the effects of sulfonamides, alkalis, water restriction and calciferol, all the patients were in the fastigium phase of excretion of calcium, i.e., in nitrogen equilibrium or positive nitrogen balance, and on full nutritive diets containing 16–18 grams nitrogen, approximately 2000 mgm. calcium and 2300 mgm. phosphorus per day.

Relation of Urinary Phosphorus to the Protein Catabolism Reaction

In contrast to the trend of urinary calcium which steadily rose during the period of nitrogen wastage and apparently independently of the protein metabolism, the phosphorus content of the urine invariably reached a peak at the height of the protein catabolism reaction and thereafter tended gradually to fall, paralleling the output of nitrogen in the urine. This tendency was manifested in all the patients and was independent of their type of diet. All the diets were constant during the periods of negative nitrogen balance. Representative examples follow:

(1) Jo., diet 1500 calories, nitrogen 7 grams, calcium 250 mgm., phosphorus 600 mgm. The urine contained an average of 1150 mgm. phosphorus during the first 10 days when nitrogen wastage was greatest; thereafter there was a gradual fall to 750 mgm. phosphorus in the urine at the 30th day when nitrogen equilibrium was reached.

(2) Ra., diet 2700 calories, nitrogen 19 grams, calcium 1600 mgm., phosphorus 2200 mgm. Urinary content of phosphorus was greatest (1450 mgm.) at the height of the nitrogen wastage period (third to ninth day), gradually falling to 1250 mgm. at the 25th day when nitrogen equilibrium was reached.

(3) Fr., diet 2300 calories, nitrogen 17 grams, calcium 2000 mgm., phosphorus 2200 kgm. Urinary content of phosphorus per day was greatest (2000 mgm.) when urinary nitrogen was greatest (sixth, seventh and eighth days). Both before and after this period of three days the daily urinary phosphorus fluctuated but little from 1500 mgm.

(4) Ha., diet 1700 calories, nitrogen 11.5 grams, calcium 275 mgm., phosphorus 1000 mgm. At the height of the protein catabolism reaction, the urinary phosphorus content was 1400 mgm. per day and fell gradually to 1100 mgm. when nitrogen equilibrium was reached.

(5) Wa., diet 1700 calories, nitrogen 11.5 grams, calcium 275 mgm., phosphorus 1000 mgm. Peak excretion of phosphorus in the urine (1450 mgm. per day) coincided with maximal nitrogen loss. There was a gradual fall in urinary content of phosphorus to 925 mgm. per day when nitrogen equilibrium was reached.

Thus, Ra. and Fr. who were given the largest diets during the period of nitrogen wastage (greater in calories, nitrogen and phosphorus), manifested less fall in urinary phosphorus excretion as nitrogen wastage lessened than did those patients on the smaller diets; although at the peak of the nitrogen wastage the diet seemed to affect the urinary excretion of phosphorus but little.

Only one observation was made on the effect of raising nitrogen and phosphorus in the diet without coincidently raising the dietary calcium. This was in the case of Je. (Table VIII); with dietary increments of 7 grams nitrogen and 500 mgm. phosphorus, an increase of 200 mgm. phosphorus in the urine occurred. With further addition of 8 grams nitrogen and 250 mgm. phosphorus, 100 mgm. more phosphorus appeared in the urine.

In the other instances when the phosphorus content of the diet was radically altered, (Table VIII) the calcium content of the diet was coincidently increased or decreased. The table demonstrates that in every instance a rise or fall in dietary phosphorus is reflected by a rise or fall in urinary phosphorus. In Jo. there was as much as 500–600 mgm. additional phosphorus in the urine in response to the addition of 1500 mgm. phosphorus to the diet, with reversal when the dietary phosphorus was lowered. In patient Bu., with similar dietary alterations, there was even greater reduction reflected in the urinary phosphorus. However, in other cases the urinary phosphorus was much

TABLE VIII

PATIENT	DAYS AFTER INJURY	CALORIES PER DAY	DIET N	DIET Ca	DIET P ANALYZED	URINE Ca	URINE P	N BAL.	CHANGE IN N INTAKE	CHANGE IN DIET Ca	CHANGE IN URINE Ca
			gm./day	mgm./day	mgm./day	mgm./day	mgm./day	gm./day	gm./day	mgm./day	mgm./day
Je.	34–36	3000	20	2000	2450	540	1350	+4			
	37–42	3680	27	2000	2950	635	1590	+5	+7	0	+95
	43–47	3680	26	2000	2950	560	1570	+3	+1	0	−75
	48–52	3680	34	2000	3200	585	1660	+10	+8	0	+25
Wh.	9–11	1800	11	300	1000	450	830	−3			
	12–14	1900	12	300	1000	525	955	−1	+1	0	+75
	15–17	1800	12	300	1000	565	875	−1	0	0	+40
	18–20	1800	13	300	1000	575	800	+1	+1	0	+10
	21–23	2500	17	2000	2200	540	915	+3	+4	+1700	−35
	24–26	2500	18	2000	2200	630	980	+1	+1	0	+90
	27–29	2500	18	2000	2200	545	1035	+1	0	0	−85
	30–32	2500	19	2000	2200	565	1145	+3	+1	0	+20
Ha.	34–36	1600	12	300	1000	425	1060	−5			
	37–39	1750	12	300	1000	405	995	−4	0	0	−20
	40–42	2750	17	2000	2100	520	1240	−2	+5	+1700	+115
	43–45	2700	16	2000	2200	465	1295	−1	−1	0	−55
Jo.	31–33	1500	8	200	600	495	670	−4			
	34–36	1600	7	200	600	485	825	−4	−1	0	−10
	37–39	2450	16	2000	2100	620	1370	+1	+9	+1800	+135
	40–42	2600	17	2000	2200	510	1340	+1	+1	0	−110
	43–45	2400	16	2000	2200	510	1350	0	−1	0	0
	46–48	2600	16	2000	2200	490	1270	+2	0	0	−20
	49–51	1700	12	250	1000	440	820	−1	−4	−1750	−50
	52–54	1700	11	250	1000	460	825	−2	−1	0	+20
Bu.	77–79	2700	17	1950	2300	280	1450	0			
	84–86	2700	16	1950	2200	230	845	+3	−1	0	−50
	91–93	1750	12	550	1000	235	745	+1	−4	−1400	+5
	98–100	1600	12	550	1000	230	750	0	0	−50	−5
Ne.	39–41	1800	12	300	1000	345	915	−2			
	42–44	1800	12	300	1000	340	845	−1	0	0	−5
	45–47	1700	11	2000	1000	475	625	−4	−1	+1700	+135
	48–50	1800	11	2000	1000	415	535	−3	0	0	−60
	51–52	1800	11	2000	1000	455	515	−1	0	0	+40
	53–55	1700	11	2000	1000	435	455	−1	0	0	−20
	56–58	1800	12	500	1000	355	655	0	+1	−1500	−80

TABLE VIII—*Continued*

PATIENT	DAYS AFTER INJURY	CALORIES PER DAY	DIET N	DIET Ca	DIET P ANALYZED	URINE Ca	URINE P	N BAL.	CHANGE IN N INTAKE	CHANGE IN DIET Ca	CHANGE IN URINE Ca
			gm./day	*mgm./day*	*mgm./day*	*mgm./day*	*mgm./day*	*gm./day*	*gm./day*	*mgm./day*	*mgm./day*
Ne.—	59–61	1600	11	500	1000	255	805	−2	−1	0	−100
Cont.	62–64	2000	12	300	1000	260	1060	−2	+1	−200	+5
	65–67	1900	12	300	1000	305	655	−3	0	−200	+45
Be.	5–8	2000	11	1400	1500	510	1510	−7			
	8–14	2200	13	325	1100	580	1190	−4	+2	−1075	+70
	14–20	2400	13	2000	1800	575	1300	−1	0	+1675	−5
	20–26	3300	20	2000	2200	570	1350	+5	0	0	−5
	26–32	2200	11	325	950	535	1000	0	−9	−1675	−35

less affected by dietary shifts of a similar order of magnitude. These observations on coincident calcium and phosphorus changes in the diets were all made after nitrogen equilibrium or positive nitrogen balance had been established.

In the case of Ne., on a constant phosphorus intake, the addition of 1700 mgm. calcium as calcium lactate caused a reduction of urinary phosphorus of approximately 200–300 mgm. per day.

In evaluating the influence of extraneous factors, such as dietary changes, upon the total urinary excretion of calcium and phosphorus over 24-hour periods, certain obvious facts should be kept in mind: (1) Even on fixed constant diets, of all types used in these experiments, the daily output of these elements in the urine fluctuated to some extent (up to 100 mg. or even more), due to other influences as yet not determined. Therefore, a dietary change must uniformly effect an alteration of more than 100 mgm. per day in the urinary calcium or phosphorus output to be considered significant. (2) The patients included in this study were normal healthy persons so far as it was possible to determine. Other effects might result from similar experimental conditions in persons suffering from abnormalities of the gastrointestinal tract which might alter the capacity of absorbing food elements; in persons with renal diseases altering the excretory functions; or in patients with skeletal disease in which the movement of calcium and phosphorus into or from the bone might be markedly altered.

The results of the experimental observations reported above may be summarized as follows:

(1) After fracture or osteotomy, on constant diets of widely divergent types, during the period of immobilization of the patient in cast, the urinary excretion of calcium tends to rise until the period of general increased protein destruction is past; the amount of calcium excreted in the urine thereafter remains relatively constant for 30 to 60 days or until mobilization of the patient.

(2) Urinary excretion of phosphorus reaches its peak at the height of the protein breakdown process and falls to a low point at the end of the period of nitrogen wastage.

(3) A. During the period of maximal excretion of calcium marked increase in dietary calcium without coincidental rise in dietary phosphorus is associated with small increases in urinary calcium.

B. Increase in dietary calcium with, at the same time, increase in dietary phosphorus (in ratios Ca:P as obtained in cows' milk) results in negligible and inconsistent rise in urinary calcium.

(4) A. Coincidental increases in dietary calcium and phosphorus result in a consistent rise in urinary phosphorus.

B. Increase in dietary calcium without coincidental increase in phosphorus results in a fall in urinary phosphorus excretion.

C. Increase in dietary phosphorus without coincidental rise in dietary calcium results in rise in urine phosphorus.

Blood Constituents

Studies on the blood constituents of these patients convalescent from fracture and osteotomy revealed no changes which were of striking note. No significant changes in the hemoglobin or RBC were observed in any of the patients. No determinations of blood volume or extracellular water compartment were made on any of the patients. There was a small fall of approximately 5–8 Meq. per liter in the concentration of chloride ion of the serum observed almost uniformly during the first week after injury, but no significant alteration of serum bicarbonate accompanied this. During the first week after injury there was a general tendency for the hematocrit and serum protein concentration to fall slightly, but this observation was by no means uniform and in most instances the changes were barely outside the limits of error for the methods used.

Concentration of inorganic phosphorus in the serum showed little tendency to change. In two instances (Bu. and Wa.), both adult males whose fractures were complicated by infections, the serum inorganic phosphorus rose gradually from 4 to 5.5 mgm. per 100 cc. at the end of the first month, falling gradually back to the initial concentration during the subsequent six weeks. In both Be. and Je., who were 15 and 16 years old respectively, the serum inorganic phosphorus concentration was consistently above 5 mgm. per 100 cc. but did not fluctuate beyond the limits of error.

Alkaline phosphatase concentration likewise remained remarkably unaltered during the convalescence of these patients. In only one instance did the concentration of this enzyme exceed the normal; this patient suffered a compound femoral fracture from a bullet; on the second day alkaline phosphatase concentration was 4.4 Bodansky units, at the tenth day 12.3 units, at 19 days 15.5 and at 24 days 36.5. At the 31st day the concentration had fallen to 8.5 units where it remained for the subsequent two months of observation. No explanation of this marked deviation from the observations in all the other cases was apparent.

Concentration of calcium in the serum of these patients showed in most instances a tendency to rise slightly from the initial determinations (made generally on day 2 or day 3 after injury) as compared with determinations made at approximately 21 days later. The rises were very small, however, usually less than 1 mgm. calcium per 100 cc. serum; and many of the patients did not demonstrate any rise in serum calcium concentration whatever. In two instances serum calcium concentrations reached what was considered to be abnormally high limits, 13.5 mgm. per 100 cc. and 12.2 mgm. per 100 cc. Both these high calcium levels occurred in adolescent patients after osteotomies. In the first patient (Je.) this occurred 30 days after operation and 20 days after nitrogen equilibrium had been reached. Urinary calcium excretion was high in this patient (550–650 mgm.) per day; no evidence of reduced renal function was present, nor were any abnormal elements found in the urine at any time. Serum calcium determinations made the previous and subsequent weeks were both 11.9 mgm. per 100 cc.; the average serum calcium concentration of this patient was 11.5 during the entire period of observation. In the second patient mentioned

above (Wh.), the serum calcium concentration twice rose to 12.2 mgm. per 100 cc.; the first time 12 days after operation coincidental with reestablishment of nitrogen equilibrium and maximal excretion of calcium in the urine, the second time 60 days later. No symptoms of nephrolithiasis were present nor were abnormal elements found in the urine. This patient's serum calcium concentration fluctuated much more widely than that of any of the other patients, the range being from 10.1 to 12.2 mgm. per 100 cc., without visible relationship to any factors which we were able to discern.

Urinary Sediment

During our observations of them only two of the patients reported in this study manifested any symptoms or signs to suggest nephrolithiasis. However, x-rays were not made of all the patients after convalescence was complete, so we have no data on which to base the true incidence of stone formation in the group. One patient developed RBC and calcium casts during administration of calcium lactate (vide supra p. 301). No pain was experienced and the abnormal elements in the urine promptly disappeared with cessation of administration of the calcium lactate. The other patient had frank renal colic with RBC in the urine. Fluids were forced, symptoms promptly disappeared, and subsequent urography revealed no evidence of stone. Both of these patients were young (aet 14 and 17, respectively); both had undergone femoral osteotomy and the manifestations of lithiasis occurred in both during the plateau period of calcium excretion, approximately eight weeks after operation.

DISCUSSION

Interpretation of the physiological processes responsible for the pattern of urinary excretion of calcium after fracture and osteotomy is purely speculative at this time. Since patients with induced malarial chills, followed for 20 to 30 days at bed rest, showed no increased urinary excretion of calcium compared with their prefebrile control periods (20), it is difficult to imagine that the speed of rarefaction of the immobilized skeletal area does not play a dominant role. The speed of rarefaction would depend upon the difference in rate of resorption and of new bone formation. The studies of Barr, Detrick et al.

(21), in which healthy young males were immobilized in casts below the waist, disclosed slow progressive rise in urinary excretion of calcium on fixed diets. But at the end of the month's study, the daily amount of calcium in the urine of one of their patients had risen only from 60 to 120 mgm.; in the other there was a greater rise, from 100 to 330 mgm. Another patient, similarly studied later, was found to excrete 575 mgm. calcium daily in the urine in the fifth week of immobilization (22). The average excretion of our patients, who were less extensively immobilized than Detrick's patients, was approximately 500 mgm. per day at the end of 30 days. One would suspect that injury in some way increases the speed of rarefaction. The neurological injury which accompanies the immobilization of the paralytic limb after poliomyelitis is associated with speedy skeletal rarefaction; and excretion of as much as one gram of calcium per day in the urine has been observed in such patients (23).

For many years there has been speculation on the possible relationship between blood supply and the states of bone growth and rarefaction. Jones and Roberts (24), in an elaborate discussion of the subject, reached the conclusion that decreased blood supply results in increased density of bone, and hyperemia of the part results in skeletal rarefaction. It seems altogether unlikely that this thesis could be uniformly true for all circumstances, for reduction in blood supply is always followed by atrophy of the tissue supplied. Though little is known about the factors governing *blood flow through bone*, it is clearly established that muscular exercise, which greatly increases the blood flow through the muscles, is associated with the development of increased size and density of the contiguous skeleton.

Harris (25–26) cites many instances in which hyperemia was accompanied by greater growth of bone. For this reason he performed lumbar sympathectomy on children who had one leg shorter than the other due to poliomyelitis. Improvement in growth on the affected side was noted in most instances after the operation; but, unfortunately, no mention is made as to whether or not the operation effected any alteration in the density of the bone. His observations do show, however, that under certain circumstances, skeletal metabolism is influenced by the autonomic nervous system, and the presumption is that this influence is exercised through control of the blood supply.

Pearse and Morton (27) reported experimental evidence that hyperemia, induced by venous obstruction, accelerated fracture healing in dogs. There was found, in every instance on the side having the vein ligated, earlier callus formation, earlier calcification of callus and earlier union. The mechanism whereby the venous obstruction effected beneficial effects was not clear. It seems possible that the maintenance of dilated capillaries in the area, insensitive to contraction by usual stimuli such as sympathetic stimulation, cold or even adrenalin (28), concomitant with the greater lymph flow, may have promoted better bone growth.

If, under the circumstances of convalescence from fracture and osteotomy of our experimental observations, the pattern of urinary calcium excretion is related to rarefaction of the immobilized part, and if the status of the blood supply to that part plays an important role in the speed of the skeletal rarefaction, a plausible hypothesis may be advanced for the observed pattern of calciuria. The early hyperemia of the inflammatory reaction about the fracture site may be a major reason why the urinary excretion of calcium is at first normal and only gradually rises. The local inflammatory reaction following osteotomy is probably considerably smaller than that after fracture and wanes much more rapidly, as perhaps reflected in the shorter and less vigorous protein catabolism. Reflexes may be set up by the injuries, which, after the inflammatory hyperemia has passed, reduce the blood supply more than the amount of reduction that would occur from immobilization in cast alone. It has been suggested that the reaction of increased protein catabolism is related closely to, if not initiated by, absorption of products of tissue necrosis (29). The inverse relationship between the nitrogen turnover and urinary excretion of calcium would suggest that the speed with which maximal calcium excretion is reached in an immobilized patient is also partly dependent upon the degree of local trauma.

SUMMARY

The pattern of urinary excretion of calcium and phosphorus is described in patients immobilized in extensive casts after fracture of femur, tibia and after femoral osteotomy.

Urinary excretion of calcium rises steadily until the period of nitrogen

wastage ends, after which it remains fairly constant for a prolonged period. This sustained excretion of calcium via the urine was but little influenced by dietary changes, administration of calciferol or alkalis.

The possible relationship between the blood supply to the immobilized part and the pattern of urinary calcium excretion is discussed.

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COMPARISON OF ANTITHYROID ACTION OF THIOUREAS AND ALLIED SUBSTANCES

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Astwood (1) found that several derivatives of thiourea exerted a significant antithyroid action. Most of his attention was focused on thiouracil, since this substance had the most goitrogenic effect of any substance that he tested. The accumulated clinical data have demonstrated that thiouracil is very effective in controlling thyrotoxicosis, but the frequency and the significance of certain toxic reactions, notably granulocytopenia, have caused uneasiness during its use. Therefore, we have conducted studies on many thiourea derivatives and allied compounds, with the hopes of (a) securing a substance with a greater antithyroid action than that of thiouracil, thereby permitting smaller doses which, in turn, might make toxic reactions less likely; or (b) obtaining a substance which, although possessing no greater antithyroid action than thiouracil, was less toxic.

CHEMICALS STUDIED

In the search for a compound with one of the foregoing requisites, many substances[1] were studied. The open-chain thioureas were studied because of the fact that most of the substances known to induce agranulocytosis are ring structures. Some of the thioureas had one or more of the hydrogen atoms replaced with aliphatic or cyclic radicals; some had radicals attached to the sulfur. Sometimes the sulfur

[1] Most of the substances tested were kindly supplied by E. R. Squibb & Company, New Brunswick, New Jersey, through the courtesy of Dr. G. A. Harrop and Mr. W. A. Lott. Tetramethylthiourea was donated by Dr. R. O. Roblin of the American Cyanamid Company, Stamford, Connecticut. Dr. S. M. Hardy and Dr. B. W. Carey of the Lederle Laboratories, Pearl River, New York, contributed the thiouracil. Dr. R. A. Harte, of the Arlington Chemical Company, Yonkers, New York, supplied the benzylthiouracil and phenylthiouracil. We are very grateful to these individuals and their companies for the generous aid which they have given us.

of thiourea was incorporated in a ring structure. A few compounds had one of the amino groups of thiourea replaced with other radicals. One dithiourea was studied.

Other compounds investigated consisted of thiourea incorporated in ring structures. The chief substances in this group were thiohydantoins, thiouracils, and thiobarbiturates. Thioparabanic acid, thiohistidine, o-phenylene-thiourea, and acetylacetone thiourea were also tested for goitrogenesis.

A study was made of several organic sulfur compounds which did not contain the thiourea structure.

METHODS

The antithyroidal activity of the various drugs was estimated by determining their goitrogenic effect in rats, using criteria similar to those of Astwood (1). The weights of the thyroid glands were expressed in terms of the body weight of the rats. The amount of cellular hyperplasia of the thyroid cells and the disappearance of colloid were estimated. The rats were of the Wistar strain, maintained on a diet of standard rat chow. Male animals were selected in most cases, weighed about 40 grams at the beginning of the experimental period, and were caged in groups of 3 or 4. The temperature of the room was usually about 70°F. but occasionally deviated approximately 10 degrees above or below this level.

The chemicals to be tested were made up in concentrations of from 0.001 to 0.1 per cent in water. Some of the substances were not very soluble, but in certain instances the solubility was increased by making the solution alkaline. The solutions were placed in 120 cc. bottles of identical configuration. In the top of each bottle was a one-hole rubber stopper through which a piece of 7 mm. glass tubing was placed, the end being flush with the stopper. The lumen of the other end of the tube was narrowed to a diameter of about 2 mm. The bottle containing the solution to be tested was then shaken and inverted. In the case of the partially soluble drugs there was gravitation of some of the insoluble material. However, in the process of drinking the solution, some stirring was produced by the bubbles of air which ascended the column of fluid. The frequent shaking of the bottles by hand and the frequent refilling of them with the test solution,

together with the gravitation of the undissolved compound through the glass tube, made the intake of the chemical fairly uniform. With only a few exceptions, noted in the tables, the animals were given the compounds for a period of 14 days, at the end of which time they were killed by a blow on the head. The rats were weighed and the heart was immediately incised, in order to reduce the amount of blood in the field to be dissected in the neck. In some of the rats receiving thiouracil, tetramethylthiourea, and diethylthiourea, estimations of the concentrations of these substances in the blood were made (2, 5). By means of a dissecting microscope the thyroid glands were rapidly removed from the surrounding structures, gently blotted on moist filter paper and then dropped in weighing bottles containing mineral oil. Only about one minute was required from the time of cutting the muscles overlying the thyroid until the gland had been submerged in oil. It was then quickly weighed on a microbalance by an assistant. The glands were withdrawn from the oil, and the latter was largely removed by blotting with dry filter paper and sometimes by washing with ethyl alcohol. The tissue was fixed in Zenker's solution for a few hours, later mounted in paraffin, and stained with eosin and methylene blue.

Sections for microscopic study of the liver, spleen, kidneys, adrenals, pancreas and gonads were taken from 1 to 4 of the animals in the case of each of the chemicals listed in the tables. Sections of the bone marrow were taken only from the animals receiving compounds that caused distinct enlargement of the thyroid gland.

About 10 substances were tested at a time, using 3 or more rats for each test. Compounds exerting a goitrogenic effect in concentrations of 0.1 per cent were then tested in greater dilutions, in order to test their relative effectiveness.

RESULTS

It is to be emphasized that very few of the substances studied were tested in concentrations greater than 0.1 per cent, and, therefore, statements applying to the goitrogenic effects of these compounds do not necessarily apply to greater concentrations. It should also be pointed out that the smaller the rat the larger is the thyroid gland in proportion to the body weight (Fig. 1). For the most part, the

changes in the weight of the thyroid were in accord with the changes in cell size and in the amount of colloid. However, some glands which were of normal weight exhibited some cellular hyperplasia and a decreased store of colloid.

None of the non-thiourea sulfur compounds exerted a goitrogenic effect (Table III). Some of the thiourea derivatives exerted little or

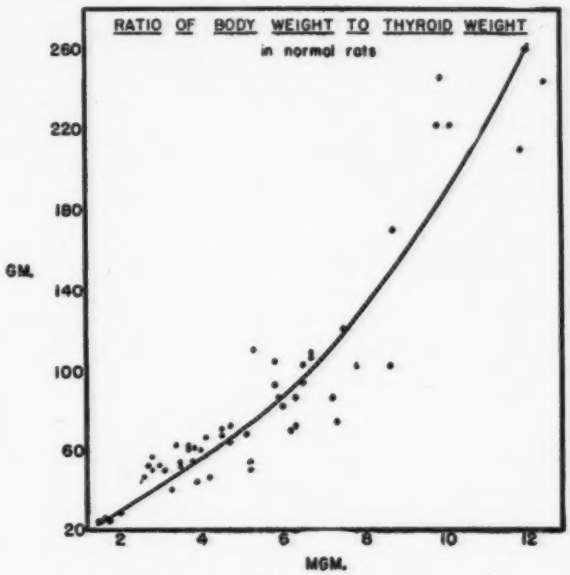

FIG. 1

no activity on the weight of the thyroid gland, on the size of its cells or on the amount of colloid in the thyroid acini (Table I). This was true of all substances with one amino group replaced by other groups, the isothioureas, and thioureas with the sulfur incorporated in ring structures.

Replacement of some or all of the hydrogens of thiourea with aliphatic or cyclic structures tended to decrease the goitrogenic activity; in most instances the substitution with cyclic radicals completely abolished the activity. Replacement with aliphatic groups caused

TABLE I

Open-Chain Thioureas

COMPOUND	FORMULA	AVERAGE VOLUME OF 0.1 PER CENT SOLUTION DRUNK IN TWO WEEKS	WEIGHT OF RATS AT DEATH		WEIGHT OF THYROID			
					Actual-grams		Mgm./100 gm. body weight	
			Range	Average	Range	Average	Range	Average
Thioureas with hydrogen atoms substituted by aliphatic radicals								
		cc.	*grams*	*grams*				
Thiourea	NH_2CSNH_2	220	60–69	65	18.3–18.4	18.4	26.6–30.6	28.6
Monodecylthiourea*	$NH_2CSNHC_{10}H_{22}$	155	70–80	75	5.0–10.5	7.2	7.1–14.2	9.7
Sym-Diethylthiourea	$C_2H_5NHCSNHC_2H_5$	67	42–56	51	3.8–8.2	6.5	9.1–14.7	12.5
Sym-Diallylthiourea	$C_3H_5NHCSNHC_3H_5$	83	66–78	72	—	3.9	—	5.4
Sym-Dibutylthiourea	$C_4H_9NHCSNHC_4H_9$	306	93–100	97	—	6.7	—	6.9
Tetramethylthiourea	$(CH_3)_2NCSN(CH_3)_2$	128	73–83	79	12.1–13.9	13.3	16.6–17.4	16.9
Thioureas with hydrogen atoms substituted by cyclic radicals								
p-aminophenyl-phenylthiourea	$NH_2C_6H_4NHCSNHC_6H_5$	183	90–106	97	—	5.2	—	5.4
Sym-Di(p-hydroxyphenyl) thiourea	$HOC_6H_4NHCSNHC_6H_4OH$	225	79–108	94	—	5.9	—	6.3
Sym-Dibenzylthiourea	$C_6H_5CH_2NHCSNHCH_2C_6H_5$	107	83–99	91	—	5.6	—	6.2
Sym-Di(O-methoxyphenyl) thiourea	$C_6H_4(OCH_3)NHCSNHC_6H_4(OCH_3)$	233	81–96	87	—	5.2	—	6.0
Thiocarbanilide	$C_6H_5NHCSNHC_6H_5$	260	103–124	111	5.7–7.2	6.3	5.4–5.8	5.7
Dibenzoylthiocarbanilide	$C_6H_5CON(C_6H_5)CSN(C_6H_5)COC_6H_5$	223	104–115	108	3.9–8.2	5.9	3.7–7.1	5.4
Thiourea from 4,4′-dichlor-2-amino-diphenyl	$C_6H_4ClC_6H_3ClNHCS—NHC_6H_3ClC_6H_4Cl$	260	121–138	130	4.4–6.0	5.3	3.6–4.3	4.0
Piperidine thiocarbanilide	$C_6H_5NHCSN(CH_2)_5$	188	65–96	83	4.4–6.2	5.7	6.5–7.4	6.9
4-Sulfonamidophenylthiourea	$NH_2SO_2C_6H_4NHCSNH_2$	106	71–79	74	—	4.1	—	5.5
Sym-Di-2-thiazolylthiocarbamide	NH(CSCHCHN)CSNH(CSCHCHN)	113	58–79	67	—	4.7	—	7.0
Diphenylthiocarbazide	$C_6H_5NHNHCSNHNHC_6H_5$	230	94–98	96	—	5.0	—	5.2
Sym-Di-o-tolylthiourea	$C_6H_5CH_2NHCSNHC_6H_5CH_3$	270	96–144	120	—	4.5	—	3.8
1-phenylthiosemicarbazide	$C_6H_5NHNHCSNH_2$	78	—	70	—	4.0	—	5.7

Sym-Di-o-tolylthiourea	$C_6H_5CH_3NHCSNHC_6H_5CH_3$	270	96–144	120	—	4.5	—	3.8
1-phenylthiosemicarbazide	$C_6H_5NHNHCSNH_2$	78	—	70	—	4.0	—	5.7

Thioureas with hydrogen atoms substituted by aliphatic and cyclic radicals

N-beta-hydroxyethyl-N-phenylthiourea	$HO(CH_2)_2NHCSNHC_6H_5$	108	63–79	70	3.3–7.0	4.7	4.8–8.9	6.6
Diacetonephenylthiourea	$C_6H_5NHCSNHC(CH_3)_2(CH_2COCH_3)$	76	81–91	86	—	5.7	—	6.6
1-beta-hydroxyethyl-2-phenylthiourea	$C_6H_5NHCSNHC_2H_4OH$	202	91–110	98	5.3–6.6	5.7	4.8–7.2	5.9
Phenylthiohydantoic acid	$C_6H_5NHCSNHCH_2COOH$	200	71–95	82	—	5.1	—	6.2
N-p-sulfonamidophenyl-N′ethylthiourea	$NH_2SO_2C_6H_4NHCSNHC_2H_5$	163	61–85	75	—	4.2	—	5.6
Dimethylcyclohexylthiourea	$(CH_3)_2NCSNHC_6H_{11}$	150	78–97	89	4.8–13.8	9.3	6.2–14.9	10.3

Isothioureas with radicals attached to the sulfur atom

Benzylisothioureahydrochloride	$NH_2C(SCH_2C_6H_5):NH\cdot HCl$	166	64–110	80	—	4.3	—	5.4
p-nitrobenzenesulfonamidobenzylisothiourea	$NO_2C_6H_4SO_2NHC(SCH_2C_6H_5)NH$	193	77–96	86	—	5.9	—	6.9
p-aminobenzenesulfonylthiotolylisothiourea	$NH_2C_6H_4SO_2NHC(SCH_2C_6H_5)NH$	242	98–107	103	3.3–4.2	3.8	3.1–4.3	3.7

Thioureas with the sulfur atom incorporated in a ring structure

1-amino-3-nitro-5-methoxybenzothiazol	$C_6H_2(CH_3O)(NO_2)NC(NH_2)S$	213	95–102	97	—	4.0	—	4.1
1-amino-5-ethoxybenzothiazole-hydrochloride	$C_6H_3(C_2H_5O)NC(NH_2)S\cdot HCl$	133	67–86	78	—	4.2	—	5.4
1-amino-5-carboxybenzothiazole-hydrobromide	$C_6H_3COOHNC(NH_2)S\cdot HBr$	225	86–115	98	—	5.8	—	5.9
1-amino-4-acetylaminopyridothiazole	$C_5H_2NH(NHCOCH_3)NCNH_2S$	88	57–64	61	2.6–4.7	3.7	4.6–7.3	6.0
Pseudothiohydantoin acetic acid	$COOHCH_2—\underbrace{CH—S—C(NH)—NH—CO}$	177	80–84	82	3.5–4.6	4.1	4.4–5.5	5.0
Pseudothiohydantoin	$\underbrace{CH_2—S—C(NH)—NH—CO}$	126	50–66	67	2.5–5.1	3.6	4.5–7.7	6.1

TABLE I—*Concluded*

COMPOUND	FORMULA	AVERAGE VOLUME OF 0.1 PER CENT SOLUTION DRUNK IN TWO WEEKS	WEIGHT OF RATS AT DEATH		WEIGHT OF THYROID			
					Actual-grams		Mgm./100 gm. body weight	
			Range	Average	Range	Average	Range	Average
Compounds with one amino group of thiourea replaced with other groups								
		cc.	*grams*	*grams*				
Dithiooxamide	$NH_2CSCSNH_2$	215	52–119	90	—	5.6	—	6.2
Tetramethylthiouramdisulphide	$(CH_3)_2NCSS_2CSN(CH_3)_2$	142	68–71	70	4.9–6.3	5.7	7.2–8.5	8.0
Thioacetamide	CH_3CSNH_2	110	62–108	85	—	4.8	—	5.6
Thiobenzanilide	$C_6H_5CSNHC_6H_5$	253	94–105	100	—	5.8	—	5.8
Dithiourea								
Ethylenediphenyldithiourea	$C_2H_4(NHCSNHC_6H_5)_2$	248	103–127	116	2.6–6.8	4.9	2.5–5.4	4.1

* Treatment given for 18 days.

less decrease in the goitrogenesis, yet sym-diallylthiourea and sym-dibutylthiourea produced no thyroid enlargement. Moreover, a dithiourea (ethylene diphenyldithiourea), was totally inactive. Tetramethylthiorea and diethylthiourea exhibited activities similar to that of thiourea.

In the group of substances with thiourea as an integral part of the cyclic structure, the thiouracils were the most active (Table III). Addition of a methyl radical in the 6-position increased the activity, as measured by thyroid weights, and the amount of colloid and cell size over that of thiouracil, whereas the addition of a phenyl radical in the same position decreased the activity (Tables II and IV). A methyl radical substituted in the 5-position of thiouracil caused a slight decrease in the goitrogenesis below that of thiouracil. The substitution of an oxygen for hydrogen in the 6-position, as in the series of thiobarbiturates, completely abolished the activity.

Thiourea incorporated in 5-membered rings, as in thiohydantoin, thioparabanic acid, and thiohistidine, was without effect, but o-phenylenethiourea was one of the most active substances tested.

Upon microscopic study of the livers, spleens, kidneys, pancreases, adrenals, and gonads no abnormalities were revealed.

DISCUSSION

During the course of this investigation it was obvious that the response of the thyroid gland varied a great deal even when dealing with littermates submitted to the same environmental conditions. This variation was consistent as far as concerned the weight of the thyroid gland, the height of the acinar cells, and the amount of colloid. The varied response in the thyroid in different rats subjected to the same treatment may result from the marked variation in the concentration of the tested compounds in the tissues and body fluids, the variations in the rate of breakdown of the compounds in the body, and in the varied rate of excretion (2–4). In the application of the experience with rats to clinical problems it must be emphasized that by adding certain chemicals to the drinking water of the rats they ingest the compound more frequently than the substances can be conveniently administered to patients. Therefore, a drug that is rapidly destroyed and rapidly excreted may be much more active in rats than in

TABLE II

Thiourea Incorporated in Ring Structures

COMPOUND	FORMULA	DAYS OF TREATMENT	AVERAGE VOLUME OF 0.1 PER CENT SOLUTION DRUNK PER RAT	WEIGHT OF RATS AT DEATH		WEIGHT OF THYROID			
						Actual—grams		Mgm/100 gm. body weight	
				Range	Average	Range	Average	Range	Average
Thiohydantoins									
			cc.	*grams*					
5[2-hydroxybenzal]-2-thiohydantoin	$\underline{NHCSNHCOC}:CHC_6H_4OH$	16	278	97–130	111	—	6.7	—	6.0
Tyrosine thiohydantoin	$\underline{NHCSNHCOCHCH_2C_6H_4OH}$	14	182	67–69	68	4.9–6.1	5.4	7.5–8.8	7.9
3-phenyl-2-thiohydantoin	$\underline{NHCSN(C_6H_5)COCH_2}$	14	113	49–73	61	1.7–3.2	2.7	3.5–5.2	4.4
Thioparabanic Acid									
Thioparabanic acid	$\underline{NHCSNHCOCO}$	14	167	73–76	74	3.2–3.7	3.4	4.4–5.1	4.7
Thiohistidine									
l,2-Thiohistidine	$\underline{NCSHNHCHCCH_2CHNH_2COOH}$	15	*	41–45	44	2.2–5.5	3.7	5.2–12.1	8.2
Thiouracils									
2-Thiouracil	$\underline{NHCSNHCOCHCH}$	13	148	65–93	81	10.2–16.9	14.3	15.7–19.4	17.6
6-Methylthiouracil	$\underline{NHCSNHCOCHCHCH_3}$	18	190	80–112	96	18.2–23.9	21.1	21.3–22.8	22.1
5-Methylthiouracil	$\underline{NHCSNHCOC(CH_3)CH}$	14	138	62–97	76	15.7–28.1	20.6	25.3–29.0	26.8
6-Phenylthiouracil	$\underline{NHCSNHCOCHCC_6H_5}$	14	280	139–179	156	12.7–16.5	15.2	8.0–12.5	9.9
Benzylthiouracil†	$\underline{NHCSNHCOCHCCH_2C_6H_5}$	14	250	137–149	142	9.5–21.6	15.0	6.9–14.5	10.5

6-Phenylthiouracil	$NHCSNHCOCHCC_6H_5$	14	280	139–179	156	12.7–16.5	15.2	8.0–12.5	9.9
Benzylthiouracil†	$NHCSNHCOCHCCH_2C_6H_5$	14	250	137–149	142	9.5–21.6	15.0	6.9–14.5	10.5
Thiobarbiturates									
2-Thiobarbituric acid	$NHCSNHCOCH_2CO$	12	160	51–58	55	2.2–4.6	3.6	4.0–8.3	6.5
5-Ethyl-2-thiobarbituric acid	$NHCSNHCOCH(C_2H_5)CO$	14	180	70–87	81	3.8–7.2	5.1	5.1–8.3	6.3
2-Thiopseudouric acid	$NHCSNHCOCH(NHCONH_2)CO\cdot\frac{1}{2}H_2O$	13	258	108–129	122	5.2–9.7	7.4	4.8–7.5	5.9
5-Thiocarbamidobarbituric acid	$NHCONHCOC(NHCSHN)CO$	13	96	54–70	64	2.4–4.5	3.8	4.4–6.7	5.8
Ammonium salt of thiocyanobarbituric acid	$NHCONHCOC(NH_4)(SCN)CO$	14	230	92–100	95	—	5.3	—	5.6
Phenylene Thiourea									
O-Phenylenethiourea	$C_6H_4NHCSNH$	18	182	74–85	78	14.3–23.9	19.3	19.3–28.0	24.7
Acetylacetone Thiourea									
Acetylacetone thiourea	$C(CH_3)CH_2C(CH_3)NCSN$	13	95	59–67	62	3.7–4.1	3.9	6.1–6.6	6.3

* The amount of thiohistidine available was limited, so this substance was given to very young rats. The substance was injected and not given in the drinking water. 0.1 cc. of a 1 per cent aqueous solution was injected subcutaneously at 9 a.m. and 1 p.m. At 6 p.m. 0.1 cc. of a 1 per cent aqueous solution containing 0.2 per cent agar was injected subcutaneously. Three animals were injected and 3 littermates served as control.

† The position of the benzyl radical is uncertain.

TABLE III

Non-Thiourea Sulfur Compounds

COMPOUND	FORMULA	DAYS OF TREATMENT	AVERAGE VOLUME OF 0.1 PER CENT SOLUTION DRUNK PER RAT	WEIGHT OF RATS AT DEATH		WEIGHT OF THYROID			
				Range	Average	Actual-grams		Mgm./100 gm. body weight	
						Range	Average	Range	Average
			cc.	*grams*	*grams*				
2-amino 4-chlorothiophenol hydrochloride	$NH_2C_6H_3(Cl)SH \cdot HCl$	16	215	107–110	109	—	7.2	—	6.6
Dithiodiglycol	$HOCH_2CH_2SSCH_2CH_2OH$	16	73	77–89	84	—	6.6	—	7.9
Ethylthioglycolic acid	$C_2H_5SCH_2COOH$	16	183	77–131	100	—	4.1	—	4.1
Thianthrene	$C_6H_4SC_6H_4S$	16	223	74–110	95	—	6.6	—	6.9
p-thiocresol	$CH_3C_6H_4SH$	16	250	77–130	101	—	5.3	—	5.2
Thiodiphenylamine	$C_6H_4NHC_6H_4S$	16	207	88–108	99	—	6.4	—	6.5
Thio-beta-naphthol	$C_{10}H_7SH$	16	288	81–110	98	—	6.9	—	7.1
Thiosalicylic acid	$2\text{-}HSC_6H_4COOH$	16	278	100–123	111	—	6.5	—	5.9
Ethylthioacetate	$CH_3COSC_2H_5$	16	230	95–121	107	—	6.7	—	6.3
2,4-diketothiazotidine	$CH_2CONHCOS$	13	128	52–71	59	2.9–6.2	4.2	5.4–8.7	6.9
2-sulfhydrylthiazolidine	$NCSHSCH_2CH_2$	11	77	37–42	38	3.8–4.2	3.9	8.9–11.5	10.3

TABLE IV

Goitrogenic Effect of Various Chemicals Administered in the Drinking Water of Rats

DRUG	AVERAGE VOLUME OF FLUID DRUNK PER RAT IN 2 WEEKS	WEIGHT OF RATS	WEIGHT OF THYROID	
			Range	Average
0.1 per cent solutions				
	cc.	*gm.*	*mgm./100 gm.*	*mgm./100 gm.*
Thiouracil	—	65–82	15.7–19.4	17.6
"	148	44–107	11.6–20.6	17.2
"	160	96–113	10.9–19.4	16.1
6-Methylthiouracil	190	80–112	21.3–22.8	22.1
5-Methylthiouracil	138	62–97	25.3–29.0	26.8
Benzylthiouracil	250	137–149	6.9–14.5	10.5
6-Phenylthiouracil	280	131–179	9.2–12.5	9.9
O-Phenylenethiourea	182	74–85	19.3–28.0	24.7
Tetramethylthiourea	128	72–83	16.6–17.4	16.9
"	—	64–75	15.3–23.9	18.3
"	—	60–71	11.5–19.3	15.2
Sym-Diethylthiourea	67	42–56	9.1–14.7	12.5
Monodecylthiourea	155	70–80	7.1–14.2	9.7
Dimethylcyclo-hexylthiourea	150	78–97	6.2–14.9	10.3
"	140	72–81	7.5–8.4	8.0
Thiourea	220	60–69	26.6–30.6	28.6
"	233	72–85	21.4–21.9	21.8
0.05 per cent solutions				
Thiouracil	150	64–73	13.0–13.3	13.2
Thiourea	185	63–87	11.0–17.0	14.2
Tetramethylthiourea	148	55–92	9.8–13.0	11.0
0.04 per cent solutions				
Thiouracil	243	110–132	13.8–26.6	18.2
"	140	56–75	4.6–15.1	9.8
"	170	79–96	9.9–19.5	14.1
6-Methylthiouracil	200	93–97	23.5–25.5	24.5
"	190	83–101	9.9–13.8	9.7
"	243	87–109	7.9–13.1	10.5
5-Methylthiouracil	107	60–65	8.8–16.2	13.0
"	160	70–80	6.0–22.4	12.4
"	143	75–90	11.5–18.8	15.2
6-Phenylthiouracil	157	75–86	10.3–11.9	10.9
Benzylthiouracil	206	85–93	6.7–9.9	7.9
O-Phenylenethiourea	157	71–89	4.1–8.9	7.3
"	147	90–98	18.6–21.6	20.0
Tetramethylthiourea	213	78–98	10.5–13.3	11.9
Thiourea	253	75–90	14.2–21.3	17.4

TABLE IV—*Concluded*

DRUG	AVERAGE VOLUME OF FLUID DRUNK PER RAT IN 2 WEEKS	WEIGHT OF RATS	WEIGHT OF THYROID	
			Range	Average
0.02 per cent solutions				
	cc.	*gm.*	*mgm./100 gm.*	*mgm./100 gm.*
Thiouracil	265	105–129	14.2–21.1	17.6
"	137	73–80	6.1–8.0	7.0
6-Methylthiouracil	237	105–140	13.6–26.0	18.5
"	210	84–98	9.5–12.4	11.0
5-Methylthiouracil	180	67–87	11.2–15.4	13.3
O-Phenylenethiourea	190	63–69	8.9–15.7	12.7
Thiourea	217	71–90	6.8–8.5	7.8
0.01 per cent solutions				
Thiouracil	147	62–79	7.1–10.5	8.5
"	278	90–117	8.3–11.7	9.7
"	170	81–89	5.4–11.1	9.1
"	217	84–97	4.8–13.2	8.2
6-Methylthiouracil	152	122–127	10.6–21.0	15.8
"	207	77–101	10.4–12.2	11.1
"	218	83–102	8.8–18.8	14.4
5-Methylthiouracil	177	86–113	9.3–14.7	12.0
"	127	65–71	9.9–15.8	12.9
"	173	78–97	7.2–9.8	8.5
6-Phenylthiouracil	270	137–186	5.3–7.9	6.6
Benzylthiouracil	287	118–151	7.1–7.5	7.3
O-Phenylenethiourea	147	52–82	3.1–6.3	6.0
"	222	88–106	5.0–22.4	12.3
Tetramethylthiourea	128	65–68	7.6–9.3	8.7
"	185	105–106	6.6–7.3	7.0
Sym-Diethylthiourea	153	49–73	3.5–5.2	4.6
Thiourea	190	70–85	7.9–8.9	8.5
0.005 per cent solutions*				
Thiouracil	193	74–84	5.3–6.2	5.8
6-Methylthiouracil	173	75–100	4.9–6.9	5.8
5-Methylthiouracil	210	59–98	5.1–7.5	6.2
O-Phenylenethiourea	177	66–104	6.1–9.6	8.1
Tetramethylthiourea	197	80–102	4.3–8.3	5.9
Sym-Diethylthiourea	197	41–91	5.2–9.0	7.1
Thiourea	190	60–71	8.2–9.6	8.4

* These substances were also tested in concentrations of 0.001 per cent but all were inactive.

patients because of its more frequent ingestion by the rats. In the case of thiouracil and thiourea, rats apparently break down the drugs faster than do patients.

Many clinical and pharmacological studies will be necessary to determine which of the active drugs is superior clinically. Our experiences with tetramethylthiourea and diethylthiourea have been reported (5). We have used 6-methylthiouracil, 5-methylthiouracil, and o-phenylenethiourea in a few cases of thyrotoxicosis with satisfactory results thus far.

SUMMARY

The effect of changing the structure of thiourea on its antithyroidal activity was studied by giving these substances for 2 weeks in a concentration of 0.1 per cent in the drinking water to rats. No goitrogenic effect was exerted by non-thiourea sulfur compounds, isothioureas, thioureas with the sulfur incorporated in ring structures, sym-diallylthiourea, sym-dibutylthiourea, dithiourea or thioureas with one amino group replaced by other groups. Substitution of one or more of the hydrogens of thiourea with aromatic radicals abolished the activity; replacement with certain aliphatic radicals decreased the activity.

When thiourea was incorporated in a 6-membered ring, as in the form of thiouracil, its goitrogenic activity in the smaller dose range tended to be increased. Addition of a methyl radical to the 6-position of thiouracil increased its activity, whereas the addition of a phenyl radical decreased it. The activity of 5-methylthiouracil was less than that of thiouracil.

The thiobarbiturates, thiohydantoins, thioparabanic acid and thiohistidine were relatively ineffective, whereas o-phenylenethiourea was markedly goitrogenic.

Our tentative conclusions place the investigated compounds in the following order of decreasing effectiveness: 6-methylthiouracil, thiouracil, orthophenylenethiourea, 5-methylthiouracil, benzylthiouracil, 6-phenylthiouracil, tetramethylthiourea, diethylthiourea, and thiourea. However, in some instances the differences in activity were not definite, because there was a great deal of overlapping in the results, both as regards the increase in weights of the thyroid glands and in the histo-

logic changes. In large doses all of these compounds exerted a marked goitrogenic effect. Moreover, the relative activity of the compounds in rats may not be the same in patients, because of the difference in the frequency of administration of the substances and because of variations in their fate.

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BRACHYMETOPODY

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Brachymetopody is a condition in which certain digits of the hand or foot appear abnormally short, due to a shortening of the metacarpals or metatarsals. It is a relatively rare condition and the majority of cases reported in the literature were isolated instances of short metacarpals. According to Stiles (1), many cases are isolated, a knowledge of the family unknown and the etiology of the condition can never be clearly determined. It may occur symmetrically or in only 1 hand or in both hands and feet. The following 3 cases appeared of sufficient interest to justify reporting.

Case 1 (*V. C. P.*): This 35 year old, single, white, male, a French-Canadian, was admitted on 14 November, 1943 because of a chronic severe metatarsalgia. Review of the *family history* revealed that his mother had a foot deformity similar to his own with shortening of the 4th metatarsal. The distal phalanges of her thumbs were abnormally short and resembled those of the patient. The patient's *past history* was not remarkable except for an episode of pain in the left knee, left testicle, left hip and left wrist in 1941. He remained in bed for 4 days and returned to work in 1 week at which time the joint complaints had entirely disappeared and the testicle had returned to normal size. The etiology of the above series of events has remained obscure. The patient denied venereal infection and mumps, and stated that the left testicle had never been injured.

The *present illness* began insidiously when the patient was 25 years old at which time he became aware of foot pain, more noticeable in the left metatarsal region. He was certain that he had never injured either foot and said he had been able to do moderately heavy work as a farmer in civilian life and had required no medical care. In August, 1942, at the time of his entry into the Army as an infantryman, he could walk 10 miles without difficulty but in a few months his feet became so painful that he could not march with the other men and was made an officer's orderly. The feet continued to trouble him, however, and he was finally hospitalized. He stated that his feet had always been "queer looking" and as far back as he could remember, the 4th toe of each foot had been short. When he was 12 to 15 years old he said he could touch his great toe against the shin bone by acute dorsiflexion.

The *physical examination* was not remarkable except for the following positive findings: The patient was a poorly developed white male who appeared younger than his stated age of 35 years. Height 66 inches. Weight 140 lbs. The right testicle was small and atrophic The left testicle was surrounded by a small varicocele. The feet were identical (see figs. 1, 2, 3). There was obvious shorten-

ing of the 4th metatarsal bones. The toes appeared to be attached to the dorsum of the foot and were pressed up into hyperextension by the toes beneath (see fig. 1). The heads of the 2nd, 3rd and 5th metatarsals appeared to be carrying most of the

FIG. 1

FIG. 2

CASE 1

Note bilateral shortening of 4th metatarsals with dorsal displacement of 4th toes, and indentation of skin between 4th and 5th toes on plantar surface of feet.

weight, which finding seemed sufficient to explain the patient's metatarsal pain There was pes planus 1° with slight pronation, marked metatarsal relaxation and adduction of the metatarsals. The toes spread widely and the heels were broad. There was a pronounced indentation of the skin 2 millimeters in depth on the

FIG. 3
CASE 1

FIG. 4
CASE 1

plantar surface of the foot between the 4th and 5th toes, forming what appeared to be a fissure 4 centimeters long (see fig. 2) There were several painless callouses on the extensor surface of each 4th toe. With relative ease the foot could be

dorsiflexed to an angle of 45° with the tibia. The toe nails were soft and brittle. The hands were of interest only in that the distal phalanges of the thumbs were abnormally short.

Laboratory work, 15th November, 1943: white blood count, 9,700. Hemoglobin, 14.5 gms. Urinalysis, Kahn and Wassermann were negative.

X-ray (see fig. 4). The examination disclosed a shortening of otherwise normal fourth metatarsal bones, each metatarsal being about 1.5 cms. shorter than the adjacent third metatarsal.

Course on the ward: There was only slight improvement in the foot pain despite the fact that the patient's activities were restricted and Thomas heels and metatarsal bars were applied to the shoes. Operative treatment was not indicated and in view of his general poor physical development and persistent complaints relative to the feet, he was declared unfit for further military service in this area.

Case 2 (R. G. H.): This 25 year old single, white, American male was admitted on the Orthopedic Service on 24 October, 1943 complaining of pain in the metatarsal region of the right foot. The patient's father, who was well acquainted with the *family history*, stated that a maternal grandmother and her sister had a short 4th toe on the right foot, and two first cousins on the same side of the family had web toes on the right foot. The *past history* was non-contributory. The *present illness* began when the patient was 10 years old at which time he observed an indentation in the skin on the plantar surface of the right foot between the 4th and 5th toes resembling a fissure and this was painful because the skin edges rubbed against one another when he walked.

His father stated, "The patient was born with a short toe on the right foot. This toe grew proportionately with the rest of the foot and was always about the length of the 5th toe or, perhaps, shorter."

The patient had noticed at the age of 10 occasional pain in the region of the longitudinal arches associated with walking and relieved by arch supports and rest. He thought that the fissure had decreased in size during the past several years. He did not remember having injured either foot. In March, 1943, 15 months after entry into the Army and associated with marching, the metatarsal region of the right foot became extremely painful. Accordingly, on 4 October, 1943 he was hospitalized and bed rest promptly relieved the foot pain.

The *physical examination* was not remarkable except for the following positive findings: The patient was a healthy, white, male who appeared his stated age of 25 years. Height 70¼ inches. Weight 182 lbs. There was approximately a 1 inch shortening of the 4th metatarsal on the right and a pes planus 2° bilateral with slight pronation, more pronounced on the right (see figs. 5, 6 and 7). The symptoms were referred to the metatarsal region on the right and weight bearing appeared to be concentrated principally on the head of the 3rd metatarsal on the right over which there was a small callous. The right metatarsal arch was relaxed. Between the 4th and 5th toes, right, there was an indentation of the skin (see figs. 6 and 7) which formed a fissure 2 centimeters long and 1 millimeter in depth similar to that observed in case No. 1 but less prominent.

FIG. 5
CASE 2

FIG. 6
CASE 2

FIG. 7
CASE 2

Laboratory work, 25 October, 1943: white blood count, 7,400. Red blood count, 4,800,000. The Kahn, Wassermann and urinalysis showed no abnormalities.

X-ray (see fig. 8). The bones of both feet appeared normal except that the right 4th metatarsal was 1.7 cms. shorter than the left 4th metatarsal.

Course on ward: The patient, when admitted to this hospital on 24 October, 1943, had been hospitalized elsewhere since 4 October, 1943 because of severe metatarsalgia, right. The pain in his right foot had improved very much with

FIG. 8

CASE 2

Only right foot resembles Case 1

bed rest. Metatarsal bars were put on his shoes and these improved the discomfort in his feet so much that he was sent back to full duty several weeks later.

Case 3 (A. J.): This 22 year old, single, white Hebrew male was admitted to this hospital on 11 January, 1944 because of rather severe pain in the feet. A review of the *family history* and *past history* was non-contributory. The patient stated that he had never had any trouble with the feet and had never noticed any abnormalities of the feet until the *present illness* which began 9 months before admission to this hospital, when, following a long hike, he began to suffer a rather severe pain along the lateral aspect of the left foot in the region of the longitudinal arch. The right foot was similarly affected but the pain was less intense. He reported on "sick-call" on a number of occasions and was finally reclassified to limited duty in a quartermaster outfit as a checker, but in spite of the fact that he was given arch supports, the foot pain persisted and he was subsequently evacuated to this hospital.

The *physical examination* was not remarkable except for the following positive findings: The patient was an obese and rather poorly developed, single, Hebrew male who appeared older than his stated age of 22 years. There was a bilateral pes planus 1°, with moderate adduction of the metatarsals. A distinct shortening of the 4th metatarsal bone on the right was apparent, and rather prominent metatarsal callouses were noted on both feet, more pronounced on the right. Symptoms were referred to the lateral aspect of the feet, the anterior aspect of the ankle joint and the metatarsal region. There was an indentation of the skin between the 4th and 5th toes on the plantar surface of the right foot, 3 centimeters in length

FIG. 9

CASE 3

Right foot closely resembles Case 1

resembling a shallow fissure, similar to that observed in cases No. 1 and No. 2, and some relaxation of the metatarsal arches of both feet.

Laboratory work, 12 January, 1944: white blood count, 9,600. Hemoglobin, 16 gms. Urinalysis, Kahn and Wassermann were negative.

X-ray (see fig. 9), 20 January, 1944. The bones of the feet were normal except for the right 4th metatarsal which was 1 c.m. shorter than the left.

Course on the ward: There was only slight improvement in the patient's condition. Various types of arch supports were tried, but in view of his generally poor physical development and the persistent but mild foot discomfort, he was considered unfit for further military service in this area.

DISCUSSION

The etiology of individual abnormally short metatarsals has been the subject of controversy. Chrysopathus (2), in 1921, favored the

infectious theory as the cause of short metatarsals and suggested that tuberculosis in early childhood might be the cause which, not being very active, was not observed during its first stages and subsequently healed, damaging the growth centers of the bone. The X-rays and history in these 3 cases which were examined in this hospital did not support his thesis. Steggerda (3), in 1942, reported 2 cases of shortening of the 4th metatarsal bones of both feet. He was of the opinion that this deformity was congenital and showed that a similar condition existed in a maternal uncle. Stiles, in 1939, reported a case of a young woman with shortening of the 4th metacarpal. He remarked that most of these cases were isolated and a knowledge of the family unknown, but he was of the opinion that it was hereditary and due to an irregular dominant. He stated that it might occur symmetrically in only 1 hand or in both hands and feet. It is of interest that the 4th metatarsal is most frequently affected. In none of these cases was there a history of trauma or infection nor could any disease such as tuberculosis be traced in the family history.

TREATMENT

Most reports do not mention the treatment of these cases as the authors are primarily interested in the relationship to heredity, and the treatment is necessarily limited. Presumably many of these cases are able to carry on through life without symptoms, but when they are subjected to strenuous physical activity such as was encountered in these cases, their feet become extremely painful. It is apparent that such assymetry of the metatarsal heads would lead to a poor mechanical foot in the metatarsal region and that treatment should be directed primarily at relieving the foot strain by means of various types of arch supports, weight reduction and limiting physical activity as much as possible.

CONCLUSIONS

1. 3 case reports on patients whose primary complaint was severe foot pain are presented because they demonstrate an interesting and rather rare condition manifested by abnormal shortening of the 4th metatarsal bones.

2. Such deformities disturb the foot mechanics and cause severe foot strain.

3. Evidence presented suggests that brachymetopody is congenital in origin.

4. The disposition of these cases was as follows:—One case was improved enough with appropriate shoe corrections to return to duty; the other two cases were returned to the zone of the interior as unfit for overseas duty.

Miss Valerie Greig, Pfc. Isadore Sclare, T/3 Peter Zaharchuk and Major George Berkheimer assisted in the compilation of the above data.

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THE MICRODETERMINATION OF ANTIMONY[1]

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The chemotherapy of several tropical diseases depends on the administration of a variety of antimony compounds. Despite this, no simple and accurate method for the estimation of antimony in biologic material has been described. As a result, the pharmacological basis for the use of antimony has been meager, and its clinical application has been largely empirical. Goodwin and Page (1) have summarized earlier analytical methods and have pointed out their deficiencies. These authors have proposed the use of the polaragraph for the determination of antimony in plasma and urine.

Frederick (2) introduced Rhodamine B (tetraethyl-rhodamine) as a reagent for the colorimetric determination of quinquevalent antimony. No provision was made, however, for the preparation of the sample. This has developed into the primary problem involved. Secondly, the technique for development of color was a precarious and difficult one, "about four percent of all analyses failing for one reason or another". This original publication, nevertheless, was the basis of succeeding work; it indicated to the present writer that this colored complex was admirably suited for analytical work. The Rhodamine B-Sb^{V} complex is highly colored (red), stable, reproducible, obeys the Beer-Lambert law, and even under visual conditions gives a suitable color in dilution of one to ten million. Arsenic does not interfere, nor do the normal constituents of tissue with the exception of large quantities of iron.

Webster (3) made a considerable advance over Frederick's original method by using benzene to extract the metal-dye complex from the

[1]Part of the work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Johns Hopkins University. This research was begun at Wallace Laboratories, New Brunswick, New Jersey, with the cooperation of Dr. Sophie L. Plechner; we also wish to thank Mr. W. H. Yeager for technical assistence. This is a preliminary report.

aqueous solution. This simplified the procedure used to develop the color; but the principal difficulty was not met by either method. This concerned the preparation of the sample, which in the case of biological material involves the destruction of all organic material present.

The usual wet-ashing technique as carried out for arsenic or mercury in biological material was unsatisfactory, since erratic losses of from 0–70% occurred. These were observed when mixtures of sulfuric acid and nitric acid or peroxide were used and also extended to cases of other oxidizing mixtures. They did not occur when sulfuric acid alone was used. This means of digestion, however, was inadequate for all but centigram amounts of tissue. Furthermore, losses were exaggerated (even if sulfuric acid alone was used) if tissue containing antimony was allowed to stand for several days (3). These losses were originally ascribed to volatilization (4). However, since arsenic, which is generally more volatile, may be digested in this manner and may also stand in tissue with no loss, this explanation seemed difficult to accept. Finally, as we have indicated, antimony could be recovered quantitatively from a high temperature wet-ashing if active oxidizing agents were absent. An investigation of the valency state, following a typical sulfuric-nitric digest, showed that about 35% was in the tivalent state, 15% in the quinquevalent state, and 50% in neither state or "lost". Dr. H. J. Magnuson (5) suggested that this unreactive portion, which could not be oxidized by ceric sulfate, was "quadrivalent" antimony. We have not isolated Sb_2O_4 from such a mixture but Conrad (6) has shown that the nitric acid oxidation of antimony is never complete to Sb^V; the result of repeated trials is a mixture of the three oxides, Sb_2O_3, Sb_2O_4, and Sb_2O_5. It is probable that similar changes take place in tissue, both *in vitro* and *in vivo*. This would explain the losses referred to above, where antimony was left in contact with tissue before analysis by the Webster method. This behavior is in sharp contrast to that of arsenic, which nitric acid converts quantitatively to the quinquevalent form. The intermediate oxide of antimony, Sb_2O_4, may be regarded as a mixed oxide, although there is some evidence that it is a chemical individual, with antimony in the rôle of Sb^{IV} (7). In either case, it is a definite and reproducible substance with particular properties, viz., resistance to oxidization under conditions as described, and susceptibility to reduction.

In the following method all the antimony in the intermediate or unreactive state is reduced to Sb^{III} by SO_2, following the destruction of organic matter by a simple wet-ashing technique. It is then ready for colorimetric estimation with Rhodamine B. The reactions for the development of the complex dye have been studied, and the procedure has been considerably altered and simplified over that of the two earlier papers.

PROCEDURE

Chemicals: All materials should be chemically pure. Sulfuric Acid (S. G. 1.84). Nitric Acid (S. G. 1.42). Orthophosphoric Acid (S. G. 1.71). Hydrochloric Acid (S. G. 1.19). Perchloric Acid, 60%. Ceric hydrogen sulfate (G. F. Smith Co.). Rhodamine B (Eastman). Benzene. Antimony metal. Sodium sulfite anhydrous. Capryl alcohol.

Reagents: All reagents are stable except the sodium sulfite.

Hydrochloric Acid, 6 N.

Ceric sulfate, .02 N. Add 1 gram anhydrous $Ce(HSO_4)_4$ to 90 ml. distilled water, add conc. H_2SO_4 dropwise until complete solution is effected, dilute to 100 ml.

Phosphoric Acid, 3 N. Make 70 ml. conc. acid up to 1 liter with distilled water.

Rhodamine B, .02%. Add .20 g. to 1 liter distilled water.

Sodium sulfite, 1%. Dissolve 1 g. anhydrous salt in 100 ml. water. Make up daily.

Standard Antimony solutions. Weigh out .1000 g. chemically pure antimony metal, and add 25 ml. conc. sulfuric acid. Heat until the metal dissolves. Cool, dilute to 1 liter with distilled water. This solution contains 100 micrograms trivalent antimony per ml. It may be diluted to give working laboratory standards of 10 and 1 micrograms per ml. These solutions are stable with respect to this procedure since the slow oxidative changes that they undergo are reversed by the reduction process as described below. The 100 microgram standard has been used for 14 months with no detectable change.

Apparatus: Erlenmeyer flasks, 50–100 ml., or preferably, Stoll egg-counting flasks (A. H. Thomas Co.).

Three-heat laboratory hot plates.

Coleman #11 Spectrophotometer; 11–100 Cuvette carrier; matched round cuvettes 19 mm. diameter #11–101. Any colorimetric or photometric system should be suitable.

Burette, pipettes, small separatory funnels, 30 ml. beakers, small test tubes.

Digestion: Weigh out blood or tissue up to 15 grams (50 ml. urine or plasma may be used) into an Erlenmeyer or Stoll flask. Add 5 ml. of conc. sulfuric acid and about 5 ml. of conc. nitric acid. Add a few glass beads or alundum chips and 1 drop of capryl alcohol. Allow the digestion to begin spontaneously; shake to

aid solution. The digestion may be left to stand at this (or any later) stage. Place the flask on the hot plate at "low". After nitric acid fumes evolve, and digestion is proceeding rapidly (1–2 hrs.), advance the heat to "medium". If charring occurs, remove from hot plate and add 1–2 ml. nitric acid. Shake for several seconds and return the flask to the hot plate. If necessary, repeat this process and finally finish the digestion at "high" heat. When the solution is colorless or very faint yellow and fumes of SO_3 are observed, remove from hot plate. On cooling, the yellow color should disappear. If it remains, more nitric acid is required. When the solution is water white, add 2 drops of perchloric acid and heat until SO_3 is evolved. This will serve as a test in addition to destroying final traces of tissue. If the solution is still water white on cooling, the process is complete. If not, more nitric is required. After the perchloric treatment, add 3 ml. water and boil until SO_3 fumes evolve. In this laboratory 20–30 samples are generally run at once and the digestion takes 4–7 hours.

Reduction: To the clear solution after digestion add 3 ml. of 1 % sodium sulfite solution. Boil until all the water is evolved and copious fumes of SO_3 appear in the flask. After this treatment, the sample is ready for color development. This should be done without delay.

Color development: It is advisable to carry out this operation in a cold water bath. The solution must not be allowed to stand after the phosphoric acid step.

Add 5 ml. of 6 N HCl to the cooled solution, following treatment with sodium sulfite. (If a yellow color develops at this stage, it is due to over 500 gamma Fe^{III}. This will be encountered in the analysis of whole blood or red cells.) Add 2 drops of .02 N ceric sulfate, shake, and let stand for two minutes. Add 8 ml. of 3 N phosphoric acid and 5 ml. of .02% Rhodamine B. Shake the flask and cool to room temperature if necessary.

Pour the solution into a separatory funnel, and add 10 ml. of benzene to the flask. Add this to the solution in the separatory funnel. Shake 200 times. Draw off the aqueous layer and collect the benzene phase (colored red by the metal dye complex) in a dry test tube or small beaker. Let stand for several minutes to allow any droplets of water to settle, and pour off 6–8 ml. into the cuvette for estimation of color. This solution is stable in air for about an hour.

The optical density is read at 565 $m\mu$ against benzene. Using Coleman apparatus as described above (path of solution 19 mm.) 1 microgram has the value $D = .055 \pm .005$. The color of such a solution, which contains 1 part antimony in 10 million parts benzene, is sufficiently intense to be suitable for visual determination in the absence of spectrophotometric apparatus. Figure 1 relates optical density to concentration in terms of our own apparatus, constructed as described below under "Standard Reference Curve". It will be observed that the Beer-Lambert law is obeyed up to the region of 15 micrograms, and that the accuracy of an individual determination falls off considerably over 25 micrograms. If it is anticipated that this amount is present, an aliquot portion of the completed digest should be taken for color development. To this aliquot, add sulfuric acid to make the total volume 5 ml.

In the event that a very intense color is developed (D >1.1, Sb >35 micrograms) the extract may be diluted with an appropriate volume of benzene to bring the optical density within range of the reference curve. In such a case it is also occasionally necessary to perform a second extraction on the aqueous phase, using fresh benzene. The second portion is estimated in the usual way and its value added to that of the first extract. The volumes of reagents used are suitable for the determination of .5–150 micrograms in the final solution.

Standard reference curve: Using the diluted standards, add incremental values from 0 to 35 micrograms of antimony to 5 ml. H_2SO_4. Treat this with sodium sulfite solution as above, and continue with the procedure in the regular way (fig 1).

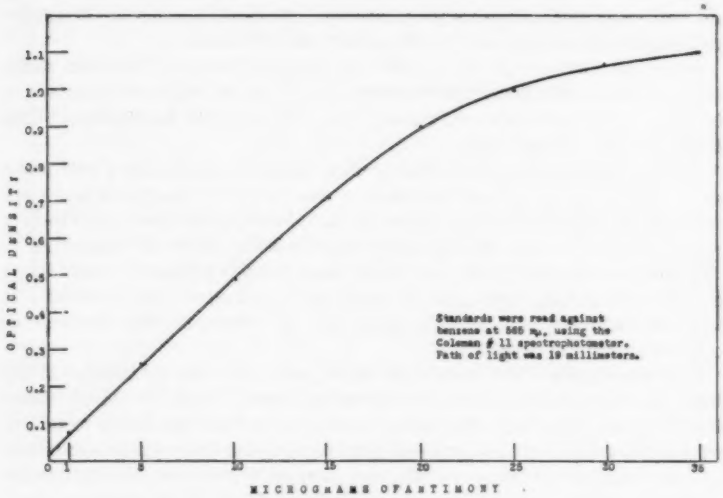

FIG. 1

The Blank: Limits: If there is less than 1 milligram of iron present (the amount in 2.5 ml. blood) the blank value is approximately 0.4 microgram. This is not significantly different from that obtained in the total absence of Fe, and since it is not additive, no correction is made on readings. Quantities of iron in excess of 1 mg. give a blank of 0.5–1.5 micrograms, and are erratic to this extent. In 5 grams of blood, therefore, it has been found expedient to subtract 1 microgram Sb from the final value. This has given satisfactory results when the above procedure has been rigidly followed. If any changes in the method are introduced, however small, it is suggested that the "iron blank" be determined directly by the digestion of 5 cc. whole blood. In the absence of Fe (or less than 1 mg.) the limit of the method is 0.5 microgram, and in the presence of 1–5 milligrams Fe

the limit is approximately 1.5 micrograms. On a series of digestions of blood (5 cc.), tissue, and urine with known amounts of antimony present, the extreme of variation encountered was ±0.3 microgram, over the 1–10 microgram range.

It is possible to extract the quantities of iron which may limit the accuracy of determinations of very small amounts of antimony. Quantitative aspects of such a procedure are now being studied.

DISCUSSION

The present method should be suitable for the rapid microdetermination of antimony in a wide variety of materials. That described here concerns biological tissue. The technique is within the general limits of accuracy for colorimetric work (8). Specifically, it provides

TABLE I

Clinical Blood Levels of Antimony

PATIENT	DRUG AND ROUTE	DOSE, MG. Sb/KG.	MICROGRAMS Sb PER ML. BLOOD AT			
			5 min.	15 min.	30 min.	1 hr.
H. F., female.....	Tartar emetic, intravenous	.40	1.8	1.3	1.1	.63
F. W., female.....	Tartar emetic, intravenous	.61	1.6	1.5	.9	.7
J. R., male.....	Tartar emetic, intravenous	.60	1.4	.9	.82	.75
M. B., female.....	Anthiomaline, intramuscular	.50	.62	1.2	1.1	1.0

for the estimation of 1–150 micrograms of antimony in blood or tissue up to 15 grams, and in urine or serum up to 50 grams.

This method has proven satisfactory in the determination of antimony in all the tissues of experimental animals, and also in the recovery of antimony from blood following its administration at the therapeutic level in man. Table I shows whole blood levels of patients at the Johns Hopkins Hospital undergoing treatment for granuloma inguinale.[2]

It is evident that the antimony content of blood may readily be determined, and that antimony is not rapidly eliminated from the blood. These subjects were ambulatory patients, and sampling at longer intervals was not feasible. Those data are amply confirmed by blood levels obtained in the laboratory on rabbits and dogs. In

[2] Based on 3 ml. of blood, withdrawn from the vein, into dried oxalate. We wish to thank Dr. Virgil Scott for obtaining these samples.

these experiments it has been found that 6 hours after administration of trivalent antimony .2–.4 microgram per ml. are present in blood, and that smaller quantities are still detectable after 24 and 48 hours.

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